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1310-Pos Board B80
Multifunctional Aspects of PduA Shell Protein from the Microcompartments of Salmonella enterica
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Bacterial microcompartments (BMC) are a functionally diverse group of proteinaceous organelles that confine specific cellular reactions within a protein-based shell. The propanediol utilizing microcompartment (Pdu BMC) contains the reactions for metabolizing 1,2-propanediol in Salmonella. The Pdu shell is assembled from a few thousand protein subunits of several different types. The shell proteins are all constructed by the assembly of proteins belonging to the same family of homologous BMC proteins. The shell is believed to be involved in several functions including the transport of the co-factors in and out of the BMC. However, till date no such transport mechanism has been identified in Salmonella or any other BMC. In recent times the crystal structures of few of the shell proteins of the Pdu microcompartment including PduA, PduT and PduU have been solved. PduA is a major component of the proteinaceous shell of Pdu BMC and has been implicated to be involved in diffusive transport of 1,2-propanediol. Our present report indicates several other functions of PduA including proper BMC assembly and co-factor transport. Using mutational analysis followed by a series of biochemical and biophysical techniques we have identified for the first time a mutant which grows slower than the wild type Salmonella strain on 1,2-propanediol. These results and their possible implications in Pdu BMC will be presented and discussed.

1311-Pos Board B81
Characterization of Metastable Oligomers as Subunits of Amelogenin Protein Nanospheres
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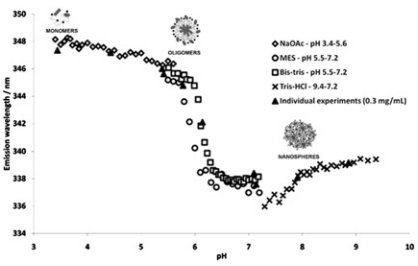
Amelogenin protein accounts for more than 90% of the protein content during tooth enamel biomineralization and controls the organized growth of enamel apatite crystals. The protein self-assembles into nanospheres in vitro. We manipulated the interactions between monomers by altering pH, temperature and protein concentration to create isolated metastable oligomers. Recombinant amelogenin (rP172), and three different mutants containing only a single tryptophan (W161,W45,W25) were used. We observed stable but unstructured oligomers with an average hydrodynamic radius (RH) of 7.5 nm at pH 5.5. Fluorescence experiments with single-tryptophan amelogenins revealed that upon oligomerization, the C-terminus is exposed at the surface of the oligomers, while the N-terminal region is involved in self-assembly. Schematics of the monomeric, oligomeric and nanosphere forms of amelogenin are indicated at the relevant pH in the figure; A plot of wavelength emission maxima of amelogenin over a range of pH values between 3.4 and 9.4. The blue-shift in emission wavelength was most apparent at around pH 6. We propose that nanospheres form via oligomers. We predict that nanospheres will break up to form oligomers in mildly acidic environments in vivo and might be functional components during enamel maturation.



1312-Pos Board B82
Effects of Meclofenamate Sodium on Proteasome Activity in Cardiac Cells
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Among the investigated causes of cardiovascular disease (CVD), the prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs) like aspirin and Meclofenamate Sodium (MS), which are used to reduce pain and fever, has been associated with increased risk of heart failure. The molecular mechanism by which NSAIDs induce cardiac dysfunction has yet to be completely elucidated, but a study using aspirin has demonstrated inhibition of proteasome activity in mouse Neuro 2a cells. The proteasome is a multi-catalytic protease complex that degrades unneeded or damaged intracellular proteins to maintain intracellular homeostasis. After 4 hours of incubation, 30μM MS was found to decrease the beta 5 chymotrypsin-like proteasome activity by 30% in H9c2 rat cardiac cells. Unlike aspirin, which was not found to directly inhibit the proteasome, MS directly modulated all three proteolytic activities of purified 20S mouse proteasome. MS significantly increased the beta 1 caspase-like and beta 2 trypsin-like activities of purified 20S proteasomes in a concentration-dependent manner. However, 500μM MS inhibited the beta 5 activity of purified 20S proteasomes by approximately 15%. Addition of 50μM MS to mouse heart homogenates decreased the 26S beta 5 proteasome activity by 20%. At MS concentrations found in the plasma of persons taking MS, MS was found to severely hinder myotube formation during C2C12 mouse skeletal myoblast cell differentiation. This is the first report of any NSAID directly affecting proteasome activity in cardiac cells and could explain why NSAIDs like MS should not be given to patients prior to heart surgery. Supported by NIH grant HL096819.

1313-Pos Board B83
The Molecular Basis of Cluster Formation by Membrane-Bound Lipidated Ras
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Recent studies found that stimulus dependent assembly and disassembly of clusters of lipid-anchored signaling proteins on the plasma membrane is a crucial mechanism by which cells achieve high-fidelity signal transmission. The best characterized examples of lipid-protein assemblies are Ras nanoclusters at the inner leaflet of the plasma membrane. Independent of expression level, 30-40% of Ras proteins assemble into 6-8 proteins per cluster. However, little is known about the physical forces underlying the domain-specific localization and clustering of these proteins. To address this issue, we carried out extensive semi-atomistic molecular dynamics (MD) simulations of the C-terminal membrane-targeting motif of H-ras (tH) in a phase-separated bilayer composed of 2000 DPPC, DLiPC, and cholesterol molecules mixed in a 5:3:2 molar ratio. We found that 4-10 tH molecules assemble into dynamic clusters whose stability varies with the extent of lipid phase separation. At ambient temperatures, the calculated cluster size distributions and the clustered fraction agree remarkably well with the available experimental data. Clusters, but not monomers or dimers, segregate to the interface between the liquid ordered and liquid disordered phases. The segregation is driven by the preferential interaction of the saturated palmitoyls in tH clusters with the similarly saturated DPPC, and of the polyunsaturated farnesyl with the unsaturated DLiPC lipids. This was confirmed by additional simulations in which individual lipid modifications were systematically removed through a process of de-palmitoylation and de-farnesylation. The preferential localization of the tH clusters at the domain boundaries resulted in a significant reduction in the line tension and changes in membrane curvature. Initial results on the full-length H-ras suggest that the same fundamental forces drive its clustering, but steric effects modulate the size and distribution of the clusters as well as the elastic properties of the bilayer.

1314-Pos Board B84
Atomic Level Supramolecular Organization and Function of an Entire Bioenergetic Organelle
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An atomic level structural model of a bioenergetic pseudo-organelle, the chromatophore, is presented along with a stochastic spatiotemporal description of its function. The chromatophore is an intracytoplasmic membrane vesicle of 50-70 nm size found in purple bacteria, comprising of over a hundred proteins that cooperate to produce ATP by converting light energy. The primary function of the chromatophore is performed through the subprocesses of photon absorption, excitation energy transfer, charge separation, diffusion of electron carriers, and the generation of a proton-motive force, culminating, finally, in ATP synthesis. These subprocesses bridge quantum mechanical and classical domains over timescales ranging from picoseconds to milliseconds. We present an atomic detail structural model of an entire chromatophore vesicle obtained by combining atomic-force microscopy, cryo-electron microscopy, crystallography, spectroscopy, and proteomics data. The chromatophore model thus constructed features 99 LH2 complexes, 15 LH1-RC dimer complexes, 7 bc1 complexes, and 1 ATP synthase, as well as over 3000 bacteriochlorophyll pigments. Based on the resulting supramolecular organization, a stochastic description of the aforementioned subprocesses are presented, revealing the principles of efficiency and robustness in the light harvesting function of the chromatophore.